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(54) Methods for generating polynucleotides having desired characteristics by iterative selective and recombination

(57) The present invention relates to a method for evolving a polynucleotide encoding a plurality of genes, e.g. multiple genes forming a multicomponent pathway. The method involves shuffling of polynucleotides by conducting a polynucleotide amplification process on overlapping segments of a population of variants of a polynucleotide encoding a plurality of genes under conditions whereby one segment serves as a template for extension of another segment to generate a population of recombinant polynucleotides. This population is screened for a recombinant polynucleotide encoding a plurality of genes having a desired property.

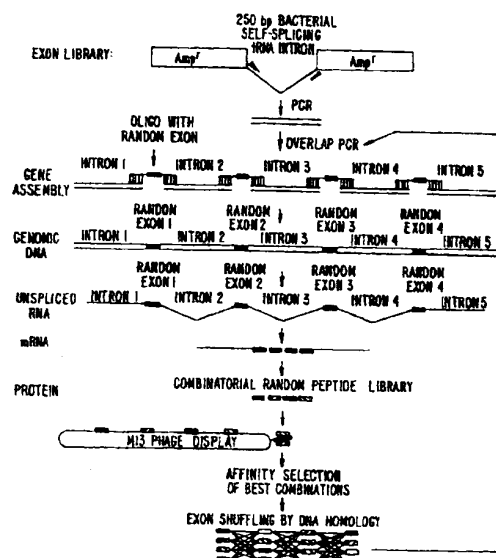


FIG. 20.



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Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.8)
Y	WO 95 22625 A (AFFYMAX TECH NV ;STEMMER WILLEM P C (US); CRAMERI ANDREAS (US)) 24 August 1995 * the whole document *	1-20	C12N15/10 C12N15/64 C12Q1/68 //C07K14/435, C07K14/545, C12N9/86, C12N9/38, C12N15/52
Y	STEMMER W: "DNA shuffling by random fragmentatio and reassembly: In vitro recombination for molecular evolution" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994, pages 10747-10751, XP002087463 * the whole document *	1-20	
Y	STEMMER W P C: "Rapid evolution of a protein in vitro by DNA shuffling" NATURE, vol. 370, 4 August 1994, pages 389-391, XP002082182 * the whole document *	1-20	
Y	W.P.C. STEMMER: "Searching sequence space" NATURE BIOTECHNOLOGY, vol. 13, June 1995, pages 549-553, XP002095510 NATURE PUBL. CO., NEW YORK, US * the whole document *	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C12Q
Y	US 5 279 952 A (WU KUN C) 18 January 1994 * the whole document *	1-20	
Y	US 5 223 408 A (GOEDEL DAVID V ET AL) 29 June 1993 * the whole document *	1-20	
-/--			
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 4 March 1999	Examiner Hornig, H
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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Application Number
EP 98 12 2014

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	R M HORTON ET AL: "Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension" GENE, vol. 77, 1989, pages 61-68, XP002090392 * the whole document *	1-20	
Y	HO S N ET AL: "SITE-DIRECTED MUTAGENESIS BY OVERLAP EXTENSION USING THE POLYMERASE CHAIN REACTION" GENE, vol. 77, no. 1, 1 January 1989, pages 51-59, XP000272761 * the whole document *	1-20	
A	WO 91 07506 A (US GOVERNMENT) 30 May 1991 * the whole document *	1-20	
A	HEIM R ET AL: "WAVELENGTH MUTATIONS AND POSTTRANSLATIONAL AUTOXIDATION OF GREEN FLUORESCENT PROTEIN" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, 1 December 1994, pages 12501-12504, XP000574454 * the whole document *	1-20	
P,X	A. CRAMERI ET AL.: "Improved green fluorescent protein by molecular evolution using DNA shuffling" NATURE GENETICS, vol. 14, March 1996, pages 315-319, XP002095449 NATURE PUBLISHING CO., NEW YORK, US * the whole document *	1-16	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Place of search THE HAGUE		Date of completion of the search 4 March 1999	Examiner Hornig, H
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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Application Number
EP 98 12 2014

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
L	WO 97 35966 A (MAXYGEN INC ;MINSHULL JEREMY (US); STEMMER WILLEM P C (US)) 2 October 1997 * examples I-IV. * ---	1-16	
T	CRAMERI A ET AL: "Molecular evolution of an arsenate detoxification pathway y DNA shuffling" NATURE BIOTECHNOLOGY, vol. 15, May 1997, pages 436-438, XP002082183 * the whole document * -----	1-16	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 4 March 1999	Examiner Hornig, H
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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ON EUROPEAN PATENT APPLICATION NO.**

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
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04-03-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9522625 A	24-08-1995	US 5605793 A	25-02-1997
		AU 2971495 A	04-09-1995
		CA 2182393 A	24-08-1995
		CN 1145641 A	19-03-1997
		EP 0752008 A	08-01-1997
		JP 10500561 T	20-01-1998
		US 5811238 A	22-09-1998
		US 5837458 A	17-11-1998
US 5279952 A	18-01-1994	NONE	
US 5223408 A	29-06-1993	US 5736135 A	07-04-1998
WO 9107506 A	30-05-1991	AU 6886991 A	13-06-1991
WO 9735966 A	02-10-1997	US 5837458 A	17-11-1998
		AU 2337797 A	17-10-1997
		AU 2542697 A	17-10-1997
		WO 9735957 A	02-10-1998
		AU 1087397 A	19-06-1997
		CA 2239099 A	05-06-1997
		EP 0876509 A	11-11-1998
		WO 9720078 A	05-06-1997

